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(21) International Application Number: PCT/US96/18550 (22) International Filing Date: 26 November 1996 (26.11.96) (30) Priority Data: 08/563,717 28 November 1995 (28.11.95) US (71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US). (72) Inventors: McKITTRICK, Brian, A.; 67 Laurel Avenue, Bloomfield, NJ 07003 (US). TULSHIAN, Deen; 4 Saddle Ridge Drive, Lebanon, NJ 08833 (US). (74) Agents: MAGATTI, Anita, W. et al.; Schering-Plough Corporation, Patent Dept. K-6-1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US).		(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: 2'-[[[4'-HALO-[1,1'-BIPHENYL]-4-YL]METHYL]-5'-METHYL-SPIRO[CYCLOPENTANE-1,7'-(8'H)-[3H]IMIDAZO[2,1-b]PURIN]-4'-(5'H)-ONES <div data-bbox="597 1192 1058 1432"></div> <div data-bbox="1136 1297 1169 1333">(I)</div>		

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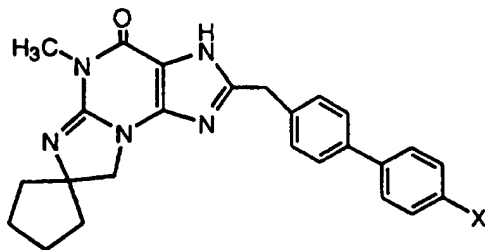
10 **2'-[[4'-HALO-[1,1'-BIPHENYL]-4-YL]METHYL]-5'-METHYL-**
SPIRO[CYCLOPENTANE-1,7'-(8'H)-[3H]IMIDAZO[2,1-b]PURIN]-
4'(5'H)-ONES

BACKGROUND

The present invention relates to 2'-[[4'-halo-[1,1'-biphenyl]-4-yl]methyl]-5'-methyl-spiro[cyclopentane-1,7'-(8'H)-[3H]imidazo[2,1-b]purin]-4'(5'H)-ones, their use in treating cardiovascular and pulmonary disorders, and pharmaceutical compositions comprising them. Phosphodiesterase inhibitory compounds of this invention were generically but not specifically disclosed in PCT publication WO91/19717, published December 26, 1991, and related compounds were generically and specifically disclosed in WO94/19351, published September 1, 1994. We have found that the compounds of the present invention show unexpectedly superior plasma levels compared to the compounds of the prior publications when administered intravenously, subcutaneously or orally.

SUMMARY OF THE INVENTION

The present invention is directed to 2'-[[4'-halo-[1,1'-biphenyl]-4-yl]methyl]-5'-methyl-spiro[cyclopentane-1,7'-(8'H)-[3H]imidazo[2,1-b]purin]-4'(5'H)-ones of the formula I:



I

or a pharmaceutically acceptable salt thereof, wherein:

X is fluoro, chloro or bromo.

- 2 -

The compounds of formula I are useful as antihypertensive, bronchodilating and blood platelet inhibiting agents. Compounds of the invention are useful in inhibiting phosphodiesterase enzymes; the inhibition of vascular phosphodiesterase is associated with vasodilation and vasorelaxation, and therefore is expected to induce antihypertensive and antianginal activity. Compounds of formula I can also serve as smooth muscle relaxants and are therefore useful in the treatment of bronchoconstriction. Such compounds also can inhibit smooth muscle proliferation, vascular growth and platelet function and are useful in treating conditions such as restenosis post angioplasty, atherosclerosis and conditions which benefit from inhibiting platelet function. Through one or more of the above physiological mechanisms, compounds of formula I are also useful in treating ischemia and peripheral vascular diseases.

Compared to previously known phosphodiesterase inhibitors of similar structure, the compounds of the present invention are not as readily metabolized. They demonstrate good selectivity of inhibition of Type I and Type V phosphodiesterase isozymes while maintaining high blood levels and demonstrating antiplatelet and vasodilator activity.

The present invention is also directed toward a pharmaceutical composition containing a compound of formula I in an amount effective to inhibit phosphodiesterase enzymes, smooth muscle proliferation, vascular growth or platelet function, or to relax smooth muscle. The present invention is also directed toward a pharmaceutical composition containing an anti-hypertensive, an anti-anginal, a bronchodilating or a platelet inhibiting effective amount of a compound of formula I.

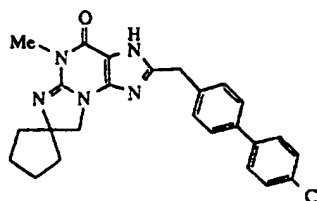
The present invention is also directed toward a method for treating hypertension, angina, bronchoconstriction, restenosis post angioplasty, atherosclerosis, ischemia, peripheral vascular diseases, or diseases benefitting from platelet inhibition in a mammal comprising administering to a mammal in need of such treatment an amount of a compound of formula I effective to treat any of the above diseases. The present invention is also directed toward a method for maintaining guanosine 3',5'-cyclic monophosphate (cGMP) levels in a mammal by administering an amount of a compound of formula I effective to maintain or increase cGMP levels.

- 3 -

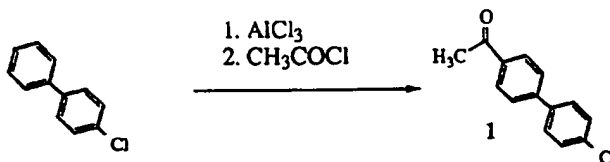
DETAILED DESCRIPTION OF THE INVENTION

Compounds of the invention have a basic nitrogen containing moiety, and can form pharmaceutically acceptable salts with organic and inorganic acids. Examples of suitable acids for such salt formation are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic and other mineral and carboxylic acids well known to those skilled in the art. The salts are prepared by contacting the free base form with a sufficient amount of the desired acid to produce a salt in the conventional manner.

The compounds of the present invention can be prepared by several routes, such as those described in WO91/19717 and WO94/19351. The following examples show typical procedures, but those skilled in the art will recognize that the preparation of these compounds is not limited to these procedures. In the examples below, Me refers to methyl, Bn refers to benzyl, Ph refers to phenyl and SEM refers to trimethylsilylethoxymethyl.

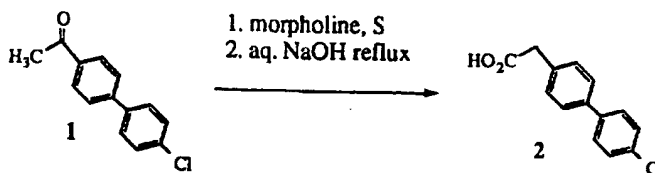
EXAMPLE 1

Step 1:

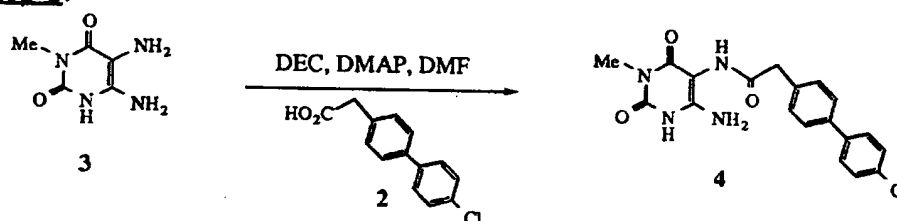


To a stirred ice-cold solution of 4-chlorobiphenyl (115 g, 0.61 mol) in CH_2Cl_2 (1200 ml) add AlCl_3 (97.5 g, 0.73 mol) in portions over 0.5 h. Add acetyl chloride (48 ml, 0.68 mol) dropwise over 0.75 h and stir the resultant red reaction mixture at 0-5 °C. After 3.5 h, allow the reaction mixture to warm to 10 °C, then pour onto a slurry of ice/1M HCl (1000 ml) with vigorous stirring. Allow the mixture to stand overnight, then wash the organic layer with sat'd NaCl, dry (MgSO_4), filter and evaporate to give a pale yellow solid. Triturate the solid with ether and collect to give the ketone 1 (122 g, 87%). ^1H NMR CDCl_3 δ 8.01 m, 2H; 7.62 m, 2H; 7.53 m, 2H; 7.41 m, 2H; 2.62 s, 3H.

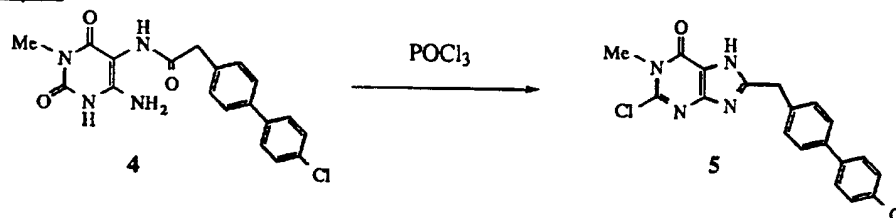
- 4 -

Step 2:

- Heat a mixture of the ketone 1 (60 g, 0.26 mol), sulfur (12.5 g, 0.39 mol) and morpholine (38 ml, 0.43 mol) at reflux. After 28 h, add the resultant brown solid to a solution of NaOH (240 g) in water (1200 ml). Reflux the mixture for 24 h, cool, collect the precipitate, wash with water and air-dry. Suspend the solid in hot water (2000 ml) and acidify to pH 2 with approximately 200 ml 1M HCl. Allow the mixture to cool, collect the solid, wash with water and air-dry. Take up the solid into EtOAc (1000 ml), wash with sat'd NaCl, dry (MgSO₄), filter and evaporate to dryness to give the acid 2 (54.9 g, 86%) as a brown solid. ¹H NMR CDCl₃ δ 7.52 m, 4H; 7.40 m, 4H; 3.72 s, 2H.

Step 3:

- To a stirred solution of the diaminouracil 3 (33.3 g, 0.21 mol), acid 2 (58.0 g, 0.24 mol) and 4-dimethylaminopyridine (2.6 g, 0.021 mol) in DMF (1000 ml), add 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (76 g, 0.26 mol). Stir overnight at room temperature, then pour the reaction mixture onto an ice water slurry (3000 ml). Collect the solid, wash with water and ethanol, and dry in vacuo to give the amide 4 (69.9 g, 87%) which is used without further purification. ¹H NMR DMSO-d₆ δ 10.51 s, 1H; 8.64 s, 1H; 7.70-7.40 m, 8H; 6.01 s, 2H; 3.65 s, 2H; 3.02 s, 3H.

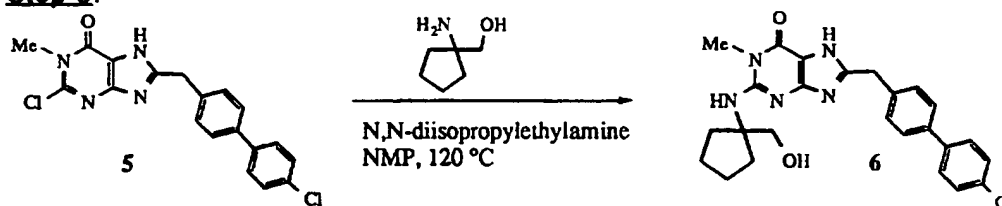
Step 4:

- Heat a stirred suspension of the amide 4 (45.0 g, 0.117 mole) in POCl₃ (1500 ml) at 90-95 °C for 43 h, then at 100 °C for 16 h, then at 110 °C for 8 h. Remove the POCl₃ by distillation at reduced

- 5 -

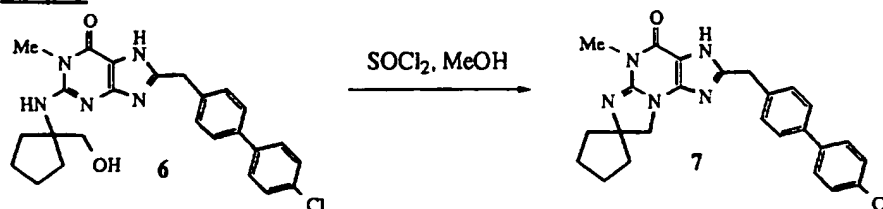
- pressure and cool the resultant oil in an ice bath. Neutralize residual POCl_3 by careful addition of saturated NaHCO_3 and collect the resultant solid by filtration, wash several times with water, air-dry, then dry in vacuo at 50°C . Triturate the solid (53 g) with 9:1 $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (1000 ml),
- 5 collect and then triturate further with 1:1 $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ with gentle heating. Concentrate the filtrate in vacuo to obtain the chloropurine 5 (25.6 g) as a brown solid. Concentrate the filtrate from the 9:1 $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ trituration in vacuo and take up in the minimum volume of approximately 4:1 $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$. Add hexanes to the resultant solution
- 10 until solids begin to separate. Collect the brown solid and wash with CH_2Cl_2 to obtain additional chloropurine 5 (6.0 g). Use the chloropurine (combined yield after drying in vacuo 31.0 g, 69%) in the next step without further purification. ^1H NMR CDCl_3 δ 7.53-7.46 m, 4H; 7.41-7.37 m, 4H; 4.31 s, 2H; 3.75 s, 3H.

15 Step 5:



- Heat a suspension of the chloropurine 5 (11.7 g, 0.030 mole), 1-amino-1-cyclopentanemethanol (7.1 g, 0.06 mole) and N,N -diisopropylethylamine (13.2 ml, 0.076 mole) in NMP (25 ml) at 120°C under argon for 30 h.
- 20 Allow the reaction mixture to cool, then pour onto a stirred slurry of ice/water (1000 ml). Collect the precipitate, wash with water, air-dry and dry in vacuo at 50°C . Triturate the solid (13.3 g) with warm 1:1 $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$, then filter. Adsorb the filtrate onto silica gel (75 g) and flash chromatograph (24:1 $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$, then 19:1 $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ when the product begins to elute) to obtain
- 25 the alcohol 6 (7.5 g, 53%) as an off-white solid. Calcd for $\text{C}_{25}\text{H}_{26}\text{N}_5\text{O}_2\text{Cl}\cdot 0.75 \text{H}_2\text{O}$: C, 62.11; H, 5.73; N, 14.49. Found: C, 62.38; H, 5.59; N, 14.30%. MS (FAB) m/z 466.1, 464.1 ($\text{M}+\text{H}$) $^+$.

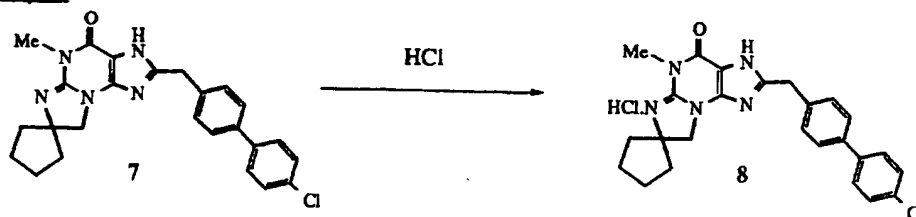
Step 6:



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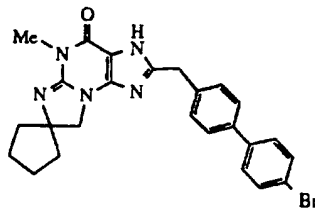
To a stirred suspension of the alcohol 6 (12.1 g, 0.026 mol) in CH_2Cl_2 (750 ml), add SOCl_2 (5.7 ml, 0.078 mol) rapidly dropwise. Stir the reaction mixture vigorously for 3 h, then evaporate in vacuo. Dissolve the residue in CH_3OH (100 ml), filter and pour the filtrate into a rapidly stirred solution of 10% NaHCO_3 . Collect the precipitate, wash with water (1000 ml) and CH_3OH (150 ml), then dry. Recrystallize from $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$ to obtain the product 7 as an off-white powder (10.30 g, 88%). Calcd for $\text{C}_{25}\text{H}_{24}\text{N}_5\text{OCl}$: C, 67.33; H, 5.42; N, 15.70; Cl, 7.95 Found: C, 67.41; H, 5.66; N, 15.41; Cl, 8.24%. MS (FAB) m/z 448.3, 446.3 ($\text{M}+\text{H}$)⁺.

10 Step 7:



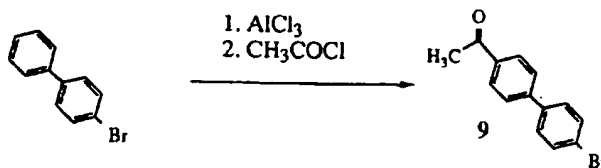
To a suspension of the tetracyclic guanine 7 (10.30 g, 0.023 mol) in EtOH (125 ml) warmed on a steam bath, add conc. HCl (1.9 ml, 0.023 mol). Filter the resultant cloudy mixture and wash the filter pad with warm EtOH (75 ml). To the combined filtrate and washings, add hot water (100 ml) and allow the solution to stand. Collect the crystalline mass, wash with 1:1 EtOH/ H_2O , air-dry and dry in vacuo at 50 °C to obtain the hydrochloride 8 (7.0 g, 63%). A second crop of product 8 (3.0 g, 27%) is obtained from the mother liquor. Combine the two crops and dry in vacuo at 50 °C over P_2O_5 . Calcd for $\text{C}_{25}\text{H}_{25}\text{N}_5\text{OCl}_2 \cdot 1.75\text{H}_2\text{O}$: C, 58.43; H, 5.59; N, 13.63; Cl, 13.80. Found: C, 58.45; H, 5.24; N, 13.45; Cl, 13.70%. ^1H NMR CD_3OD δ 7.58-7.56 m, 4H; 7.43-7.37 m, 4H; 4.38 s, 2H; 4.19 s, 2H; 3.46 s, 3H; 2.10-1.79 m, 8H.

EXAMPLE 2



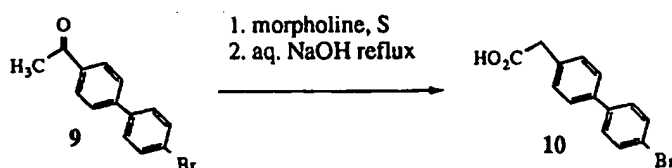
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Step 1:

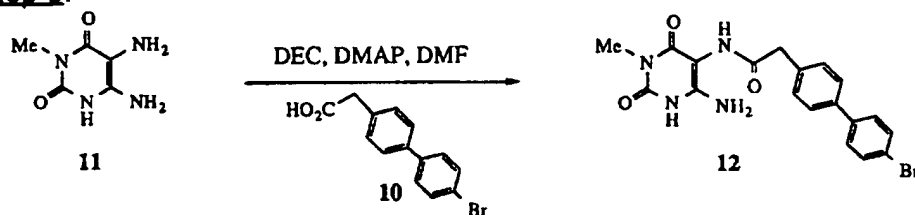


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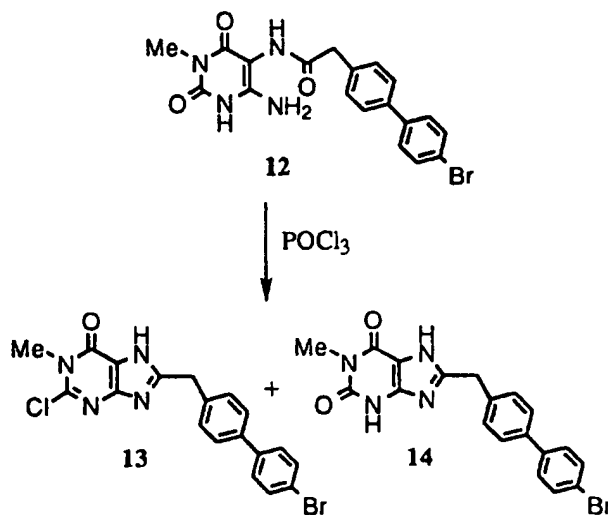
Use the procedure of Example 1, Step 1, with bromobiphenyl (51.36 g, 0.22 mol) to obtain ketone **9** (50.94g, 84%) as a light yellow solid. ^1H NMR CDCl_3 δ 8.03 dd ($J = 6.6$ and 2.2 Hz), 2H; 7.65 dd ($J = 6.6$ and 2.2 Hz), 2H; 7.55 dd ($J = 6.6$ and 2.2 Hz), 2H; 7.49 dd ($J = 6.6$ and 2.2 Hz), 2H; 2.64 s, 3H.

Step 2:

Use the procedure of Example 1, Step 2, heating with sulfur and morpholine for 4 h, to convert ketone **9** (50.93 g, 0.19 mol) to acid **10** (51.3 g, 95%), a white solid. ^1H NMR $\text{DMSO}-d_6$ δ 7.65-7.59, m, 6H; 7.35 d ($J = 8.2$ Hz), 2H; 3.60 s, 2H.

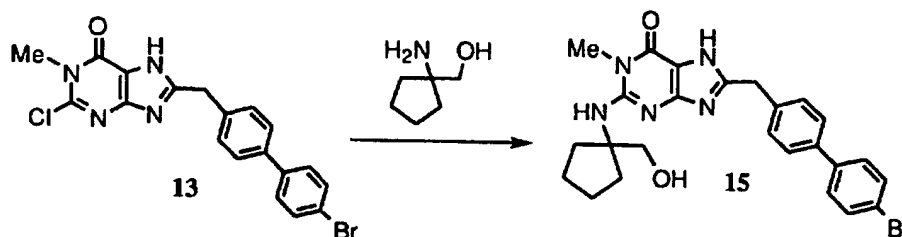
Step 3:

Treat acid **10** (51.3 g, 0.18 mol) using the procedure of Example 1, Step 3, to obtain amide **12** (61.2 g, 82%) as a yellow solid. ^1H NMR $\text{DMSO}-d_6$ δ 10.52 s, 1H; 8.66 s, 1H; 7.66-7.59 m, 6H; 7.43 d ($J = 8.2$ Hz), 2H; 6.01 s, 2H; 3.61 s, 2H; 3.04 s, 3H.

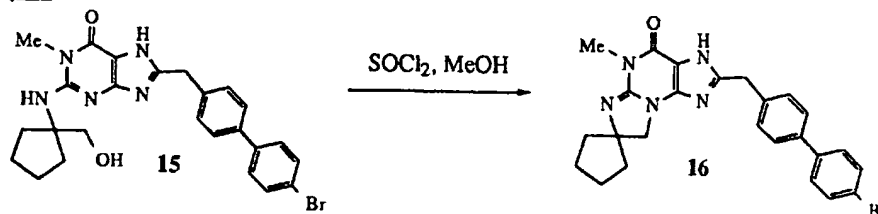
Step 4:

- 8 -

- Heat a stirred suspension of the amide **12** (40.0 g, 0.093 mole) in POCl_3 (2100 ml) at 90-100 °C for 6 days. Remove the POCl_3 by rotovaporation at reduced pressure (<45 °C) and dissolve the resultant oil in a minimal amount of CH_2Cl_2 (~400 ml). Neutralize the residual POCl_3 by carefully pouring the CH_2Cl_2 solution into cold saturated NaHCO_3 (2200 ml) over a period of 1 h. Stir the mixture overnight at room temperature, collect the resultant solid by filtration, wash several times with water, air-dry and dry in vacuo at 60 °C. The solid (28.47 g, 71%) is a 26:1 mixture of chloropurine **13** and xanthine **14**, respectively (^1H NMR analysis). ^1H NMR CDCl_3 δ 7.64-7.58 m, 6H; 7.38 d (J = 8.2 Hz), 2H; 4.13 s, 2H; 3.61 s, 3H.

Step 5:

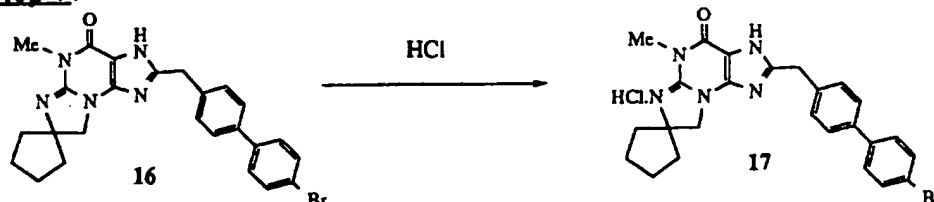
- Heat a suspension of the chloropurine **13** (28.57 g, 0.066 mole), 1-amino-1-cyclopentanol (19.77 g, 0.17 mole) and N,N -diisopropylethylamine (30 ml, 0.17 mole) in NMP (96 ml) at 120 °C under argon for 18 h. Cool the reaction mixture, then pour onto a stirred slurry of ice/brine (1000 ml). Collect the precipitate, wash with water, air-dry and dry in vacuo at 50 °C. Triturate the solid (42.36 g) with CH_2Cl_2 - CH_3OH (4:1), and then filter to give 17.48 g of a solid consisting of alcohol **15** and xanthine **14**. Adsorb the filtrate onto silica gel and subject to flash chromatography [gradient $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ = 32:1 to 19:1 (product begins eluting) to 11.5:1] to obtain alcohol **15** (7.4 g, 22%) as an off-white solid and impure **15** (5.79 g) containing xanthine **14**. ^1H NMR CDCl_3 δ 7.64-7.58 m, 6H; 7.37 m, 2H; 5.76-5.63 m, 1H; 5.09, 4.89 t,t (J = 5.8 Hz), 1H; 4.02, 3.97 s, s, 2H; 3.64 d (J = 5.8 Hz), 2H; 3.36 s, 3H; 2.05 m, 2H; 1.72 m, 4H; 1.53 m, 2H.

Step 6:

- 9 -

Treat alcohol **15** (10.1 g, 0.020 mol) according to the procedure of Example 1, Step 6, to obtain tetracyclic guanine **16** (9.6 g, 95%) as a white solid. ^1H NMR DMSO- d_6 δ 7.65-7.58, m, 6H; 7.37, d (J = 8.2 Hz), 2H; 4.02 s, 2H; 4.80 s, 2H; 3.18 s, 3H; 1.75-1.62 m, 8H.

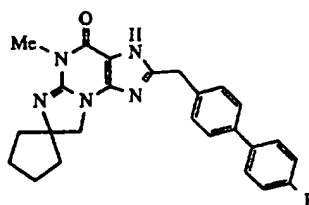
5 **Step 7:**



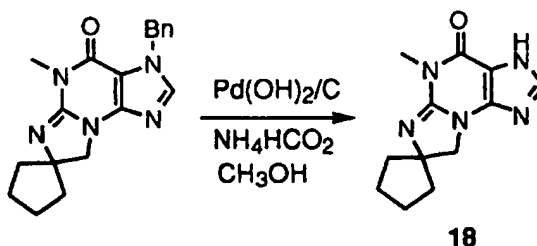
Using a procedure similar to Example 1, Step 7, except recrystallizing twice from CH_3OH and then twice from ethanol-water, treat the amine **16** (11.7 g, 0.024 mol) to obtain the hydrochloride **17** (10.22 g, 81%) as a white solid. Calcd for $\text{C}_{25}\text{H}_{25}\text{N}_5\text{OBrCl}\cdot 3\text{H}_2\text{O}$: C, 51.69; H, 5.39; N, 12.06; Br, 13.75; Cl, 6.10. Found: C, 51.58; H, 5.63; N, 11.84; Br, 13.55; Cl, 5.77%. MS (FAB) m/z 490.1, 491.1 ($M+H$). ^1H NMR DMSO- d_6 + D_2O δ 7.63-7.56, m, 6H; 7.37, d (J = 8.2 Hz), 2H; 4.28 s, 2H; 4.12 s, 2H; 3.31 s, 3H; 1.98-1.87 m, 4H; 1.79-1.61 m, 4H.

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EXAMPLE 3



Step 1:



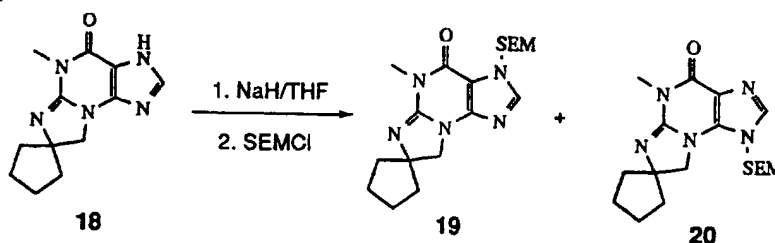
To a suspension of $\text{Pd}(\text{OH})_2$ (5g) in CH_3OH , add HNNH_4CO_2 (14 g, 223 mmol) followed by a solution of 5'-methyl-3'-(phenylmethyl)-spiro[cyclopentane-1,7'(8'H)]-[3'H]-imidazo[2,1-b]purin-4'(5'H)-one (15 g, 44.7 mmol) in CH_3OH (total volume 1000 ml) at room temperature. Heat the mixture at reflux for 6h, filter and concentrate the filtrate to obtain a white solid. Stir the catalyst in hot 10% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$, filter and concentrate. Combine the concentrated filtrates, dissolve in 10%

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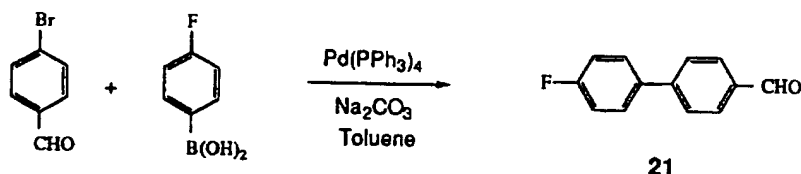
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- 10 -

CH₃OH/CH₂Cl₂ (400 ml) and wash with aqueous NaHCO₃. Extract the aqueous solution with 10% CH₃OH/ CH₂Cl₂ (200 ml x 2), dry the combined extracts over MgSO₄, filter and concentrate to obtain compound **18** as a white solid (9.7g, 88.5%). ¹H NMR CDCl₃ δ 7.62, s, 1H; 3.95, s, 2H; 3.40, s, 3H; 1.8-2.0, m, br, 4H; 1.6-1.8, m, br, 4H.

Step 2:

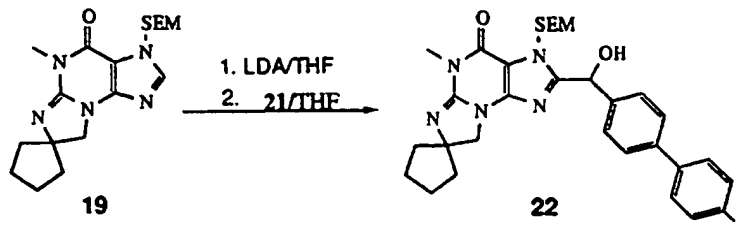
Add NaH (3.16 g, 60%, 79 mmol) to a mixture of **18** (9.7 g, 39.5 mmol) in THF (400 ml) at room temperature. Stir the mixture at room temperature for 1.5h, add trimethylsilylethoxymethyl chloride (9.88g, 59.25 mmol) and stir at room temperature for 1.5h. Quench the resulting yellow mixture with ice, extract with EtOAc (200 ml x 2), combine the EtOAc extracts and wash with brine, dry over MgSO₄, filter and concentrate. Purify the crude product by flash chromatography on SiO₂ (1% to 10% CH₃OH/CH₂Cl₂) to obtain compounds **19** (11.4 g), **20** (2.37 g) and a mixture of **19** and **20** (1.1 g) (98%). ¹H NMR of **19** CDCl₃ δ 7.61, s, 1H; 5.65, s, 2H; 3.91, s, 2H; 3.62, t, 2H; 3.38, s, 3H; 1.8-2.0, m, 4H; 1.6-1.8, m, 4H; 0.95, t, 3H; 0.00, s, 9H. ¹H NMR of **20** CDCl₃ δ 7.26, s, 1H; 5.32, s, 2H; 4.00, s, 2H; 3.53, t, 2H; 3.40, s, 3H; 1.8-2.0, m, 4H; 1.6-1.8, br, 4H; 0.92, t, 2H; 0.00, s, 9H.

Step 3:

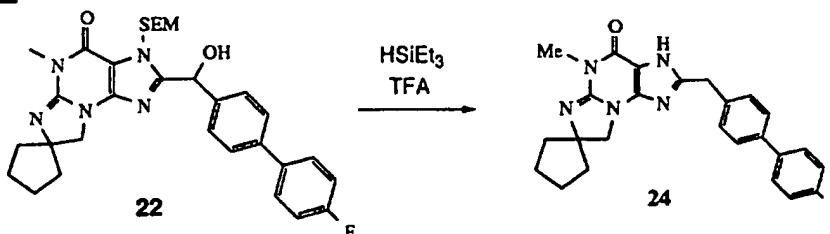
To a mixture of Pd(PPh₃)₄ (1.5 g, 1.3 mmol) in toluene (52 ml), add p-bromobenzaldehyde (4.8 g, 25.98 mmol), aqueous Na₂CO₃ (26 ml, 2.0M, 52 mmol), and a solution of p-fluorophenylboronic acid (4g, 28.58 mmol) in EtOH (13 ml) and CH₃OH (2 ml). Heat the mixture at reflux for 6h, cool to room temperature, pour into aqueous NaHCO₃, extract with EtOAc (200 ml x 3), dry over MgSO₄, filter and concentrate to obtain a dark, thick oil. Purify the crude product by flash column chromatography on SiO₂ (1:9, 1:7 EtOAc/hexane) to obtain compound **21** (4.88 g, 93.8%).

- 11 -

^1H NMR CDCl_3 δ 10.06, s, 1H; 7.96, d, 2H; 7.72, d, 2H; 7.62, dd, 2H; 7.18, t, 2H.

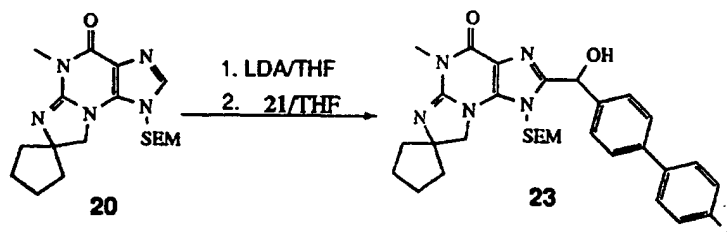
Step 4:

- 5 Add n-BuLi (6.65 ml, 2.5M, 16.62 mmol) to a solution of diisopropylamine (1.68 g, 16.62 mmol) in THF (30 ml) at 0 °C. Stir at 0 °C for 1h, cool to -70 °C and add a solution of compound 19 (4.8 g, 12.78 mmol) in THF (60ml) dropwise. After the addition is complete, stir the mixture at -70 °C for 1h, then add a pre-cooled solution of 21 (3.85 g, 19.17 mmol) in THF (15 ml). Stir the mixture at -70 °C for 3h, quench with AcOH (4 ml), warm to 0 °C and treat with aqueous NH_4Cl (30 ml). Extract the mixture with EtOAc (200 ml x 3), wash with aqueous NaHCO_3 and brine, dry (MgSO_4) and concentrate. Purify the crude product by flash column chromatography on SiO_2 (0% to 2% $\text{CH}_3\text{OH}/\text{EtOAc}$) to obtain compound 22 (5.34 g, 72.6%). ^1H NMR CDCl_3 δ 7.55, m, 4H; 7.54, 2H; 7.15, t, 2H; 6.05, d, br, 1H; 5.56, ABq, 2H; 3.97, s, 2H; 3.60, t, 2H; 3.38, s, 3H; 1.8-2.0, m, 4H; 1.6-1.8, m, 4H; 0.90, m, 2H; 0.00, s, 9H.

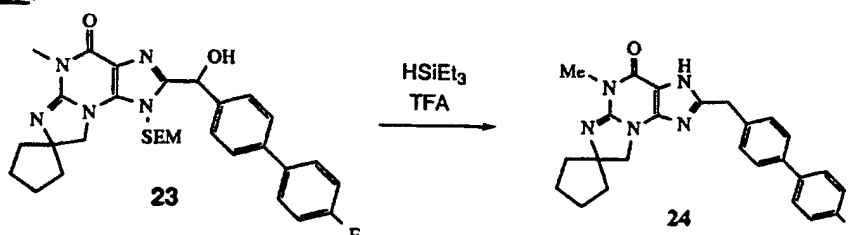
Step 5:

- 20 Add EtSiH (5.34 g, 45.4 mmol) to a solution of 22 (5.23 g, 9.08 mmol) in TFA (24 ml) at room temperature. Stir the reaction mixture at room temperature over night, carefully neutralize with aqueous NaHCO_3 , extract with 10% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$, dry over MgSO_4 , filter and concentrate. Purify the crude product by flash column chromatography on SiO_2 (3% to 10% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$) to obtain compound 24 (3.35 g). ^1H NMR CDCl_3 δ 7.51, m, 4H; 7.49, d, 2H; 7.11, t, 2H; 4.18, s, 2H; 3.92, s, 2H; 3.39, s, 3H; 1.8-2.0, m, 4H; 1.6-1.8, m, 4H.

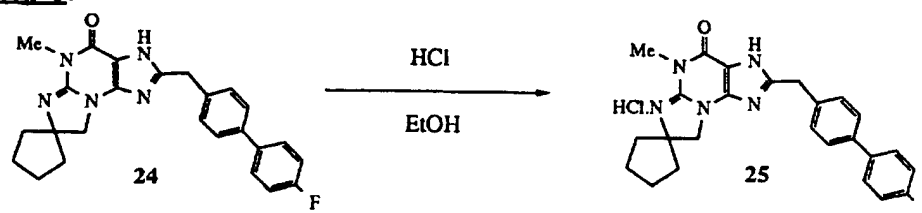
- 12 -

Step 6:

Using compound **20** (8 g, 21.7 mmol) in the procedure of Example 3, Step 4, prepare compound **23** (4.6 g, 37%). ¹H NMR CDCl₃ δ 7.50, m, 6H; 7.10, t, 2H; 6.18, s, 1H; 5.21, s, 2H; 3.99, ABq, 2H; 3.39, s, 3H; 3.10, m, 2H; 1.8-2.0, m, 4H; 1.6-1.8, m, 4H; 0.65, t, 2H; -0.10, s, 9H.

Step 7:

Treat compound **23** according to the procedure of Example 3, Step 5, to obtain compound **24**. ¹H NMR CDCl₃ δ 7.51, m, 4H; 7.49, d, 2H; 7.11, t, 2H; 4.18, s, 2H; 3.92, s, 2H; 3.39, s, 3H; 1.8-2.0, m, 4H; 1.6-1.8, m, 4H.

Step 8:

To a hot suspension of **24** (17.5 g, 40.74 mmol) in EtOH, add conc. HCl (5.1 ml, 61.2 mmol) dropwise to give a clear solution. Stir the solution at room temperature for 30 min, evaporate the EtOH to give a white solid and dry the solid in vacuo at 50°C over P₂O₅. Calcd for C₂₅H₂₄N₅OF·1.05HCl·0.75H₂O: C, 62.39; H, 5.56; N, 14.55; Cl, 7.73, F, 3.95. Found: C, 62.44; H, 5.71; N, 14.38; Cl, 7.80; F, 4.00%. ¹H NMR DMSO-d₆ δ 10.65, d, br, 1H; 7.7, m, 2H; 7.65, d, 2H, 7.40, d, 2H; 7.26, t, 2H; 4.32 s, 2H; 4.19 s, 2H; 3.39 s, 3H; 1.6-2.1 m, 8H.

Pharmaceutical Preparations

The compounds of formula I can be combined with a suitable pharmaceutical carrier to prepare a pharmaceutical composition suitable for parenteral or oral administration. Such pharmaceutical compositions are
5 useful in the treatment of cardiovascular and pulmonary disorders such as mammalian hypertension and bronchoconstriction.

The effective daily antihypertensive dose (ED₅₀) of the present compounds will typically be in the range of about 1 to about 100 mg/kg of mammalian body weight, administered in single or divided doses. The exact
10 dosage to be administered can be determined by the attending clinician and is dependent upon where the particular compound lies within the above cited range, as well as upon the age, weight and condition of the individual.

Generally, in treating humans in need of treatment for hypertension or bronchoconstriction, the present compounds can be
15 administered in a dosage range of about 10 to about 500 mg per patient generally given a number of times per day, providing a total daily dosage of from about 10 to about 2000 mg per day.

The compositions of the present invention can be administered orally or parenterally. Typical injectable formulations include solutions and
20 suspensions. Typical oral formulations include tablets, capsules, syrups, suspensions and elixirs. Also contemplated are mechanical delivery systems, e.g. transdermal dosage forms.

The typical acceptable pharmaceutical carriers for use in the formulations described above are exemplified by sugars such as lactose,
25 sucrose, mannitol and sorbitol; starches such as cornstarch, tapioca starch and potato starch; cellulose and derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and methyl cellulose; calcium phosphates such as dicalcium phosphate and tricalcium phosphate; sodium sulfate; calcium sulfate; polyvinylpyrrolidone, polyvinyl alcohol; stearic acid; alkaline earth
30 metal stearates such as magnesium stearate and calcium stearate, stearic acid, vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil and corn oil; non-ionic, cationic and anionic surfactants; ethylene glycol polymers; beta-cyclodextrin; fatty alcohols and hydrolyzed cereal solids; as well as other non-toxic compatible fillers, binders, disintegrants, buffers,
35 preservatives, antioxidants, lubricants, flavoring agents, and the like commonly used in pharmaceutical formulations.

- 14 -

Following are typical examples of oral and parenteral formulations, wherein the term "Active Ingredient" refers to a compound of formula I.

5	<u>Capsule</u>	<u>Amount (mg)</u>	
	Active Ingredient	250.0	125.0
	Lactose	173.0	86.5
	Corn Starch	75.0	37.5
	<u>Magnesium Stearate</u>	<u>2.0</u>	<u>1.0</u>
	TOTAL	500.0	250.0
10	Blend the active ingredient, lactose and corn starch until uniform; then blend the magnesium stearate into the resulting powder. Encapsulate the mixture into suitably sized two-piece hard gelatin capsules.		
15	<u>Tablet</u>	<u>Amount (mg)</u>	
	Active Ingredient	250.0	125.0
	Lactose	161.0	80.5
	Corn Starch	12.0	6.0
	Water (per thousand tablets)	120 ml	60 ml
20	(evaporates) (evaporates)		
	Corn Starch	75.0	37.5
	<u>Magnesium Stearate</u>	<u>2.0</u>	<u>1.0</u>
	TOTAL	500.0	250.0
25	Blend the active ingredient with the lactose until uniform.		
25	Blend the smaller quantity of corn starch with the water and add the resulting corn starch paste then mix until a uniform wet mass is formed. Add the remaining corn starch to the remaining wet mass and mix until uniform granules are obtained. Screen the granules through a suitable milling machine, using a 3/4 inch stainless steel screen. Dry the milled		
30	granules in a suitable drying oven until the desired moisture content is obtained. Mill the dried granules through a suitable milling machine using a 16 mesh stainless steel screen. Blend in the magnesium stearate and compress the resulting mixture into tablets of desired shape, thickness, hardness and disintegration.		
35			

- 15 -

	<u>Injectable Solution</u>	<u>mg/ml</u>
	Active Ingredient	5.00
	Methyl p-hydroxybenzoate	0.80
	Propyl p-hydroxybenzoate	0.10
5	Disodium Edetate	0.10
	Citric Acid Monohydrate	0.08
	Dextrose	40.0
	Water for injection qs. ad.	1.0 ml

10 Dissolve the p-hydroxybenzoates in a portion of water for injection at a temperature of between 60°C - 70°C and cool the solution to 20°C - 30°C. Charge and dissolve all other excipients and the active ingredient. Bring the solution to final volume, filter it through a sterilizing membrane and fill into sterile containers.

15 Biological Activity of 2'-[[4'-halo-[1,1'-biphenyl]-4-yl]methyl]-5'-methyl-spiro[cyclopentane-1,7'(8'H)-[3H]imidazo-[2,1-b]purin-4'(5'H)-ones

The present compounds are useful in inhibiting phosphodiesterase enzymes, in particular phosphodiesterase isozymes Types I and V. These phosphodiesterase enzymes are known to hydrolyze cGMP in smooth muscle. High levels of cGMP are associated with the relaxation of vascular smooth muscle, with a consequent subsequent reduction in blood pressure. Thus, it is believed that by inhibiting these phosphodiesterase enzymes, cGMP levels in muscle will be either maintained or increased, with a subsequent reduction in blood pressure. *In vivo* antihypertensive activity is determined orally in spontaneously hypertensive rats (SHR).

Phosphodiesterase inhibition in vitro:

Compounds are evaluated for inhibition of two phosphodiesterase enzymes which hydrolyze cyclic guanosine monophosphate (cGMP). The first enzyme, calcium-calmodulin dependent phosphodiesterase (CaM-PDE), is a partially pure enzyme obtained from bovine aorta homogenates and purified by DEAE-cellulose and calmodulin-affinity chromatography. The enzyme is activated several fold by Ca-calmodulin and is selective for cGMP, although it will also hydrolyze cAMP. The second enzyme, cGMP phosphodiesterase (cGMP-PDE), is a homogeneous enzyme obtained from bovine lung and purified by ion-exchange chromatography, gel filtration, and sucrose gradient

- 16 -

centrifugation. cGMP-PDE is highly selective for cGMP. Bovine aorta homogenates and primary cultures of bovine aortic endothelial and vascular smooth muscle cells contain an enzyme with properties very similar to the lung isozyme.

5 The enzyme assay is performed using a Biomek Automated Pipetting Station. Compounds are dissolved in distilled water or DMSO and diluted with 10% DMSO. Compounds are tested at several concentrations at log intervals, typically 0.1, 1.0, 10, and 100 μ M final concentration.

10 Assays contain the following components:

1 μ M substrate 3 H-cGMP

50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$

0.5 mg/ml snake venom alkaline phosphatase

0.1 μ M Calmodulin and 1 mM $CaCl_2$ (for CaM-PDE only)

15 Assays are initiated by addition of enzyme and stopped by addition of 10 mM isobutylmethylxanthine, a general phosphodiesterase inhibitor. Assays are performed for 25 minutes at room temperature to achieve 5-10% hydrolysis of substrate. The negatively charged substrates are then separated from guanosine by binding to an anion-exchange resin (AG1-X8) and centrifugation or filtration, and the product is quantitated by
20 scintillation counting in counts per minute (cpm) of the remaining soluble material. Percent inhibition is calculated as follows:

% Inhibition = $100 - [(cpm \text{ compound-blank}) / (cpm \text{ control-blank}) \times 100]$

25 Activity is expressed as the IC_{50} value, ie. the concentration required to inhibit activity of enzyme by 50 per cent.

Antihypertensive activity in rats

30 The ability of the compounds of the present invention to lower blood pressure can be assessed *in vivo* in conscious spontaneously hypertensive rats (SHR). SHR males are purchased from Taconic Farms, Germantown New York and are approximately 16-18 weeks old when anesthetized with ether. The caudal (ventral tail) artery is cannulated with polyethylene tubing (PE50) and blood pressure and heart rate are recorded as described by Baum, T. et. al, J. Cardiovasc. Pharmacol. Vol 5,
35 pp. 655-667, (1983). Rats are placed into plastic cylindrical cages where they rapidly recover consciousness. Blood pressure and heart rate are allowed to stabilize for approximately 90 minutes prior to compound administration. Compounds are administered orally as solutions or

- 17 -

- suspensions in 0.4% aqueous methylcellulose vehicle via a feeding needle. The compound or 0.4% aqueous methylcellulose vehicle are given in a volume of 4 ml/kg to SHR that had been fasted overnight. Activity is expressed as the fall in mean blood pressure (MBP) in millimeters of mercury (mm Hg). Compound-induced changes are compared with the changes in an appropriate placebo group.

Plasma levels:

- SHRs dosed orally with test compound at 10 mpk and plasma levels were subsequently measured at intervals of 0.5, 1, 2, 3 and 4 hours. Plasma levels ($\mu\text{g/ml}$) were plotted versus hours post dosing and the area under the curve (AUC, $\mu\text{ghr/ml}$) was calculated.

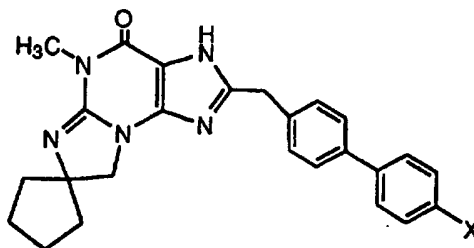
- The following test results are compared to a previously-known compound, 2'-[[[4'-methoxy-1,1'-biphenyl)-4-yl]methyl]-5'-methyl-spiro[cyclopentane-1,7'(8'H)-[3H]imidazo-[2,1-b]purin]-4'(5'H)-one (Ref. Cpd.).

Example Number	PDE IC ₅₀		SHR Antihypertensive		Plasma Level
	Type I	Type V	Dose (po)	Fall in MBP	AUC
	(nM)	(nM)	(mpk)	(mmHg)	($\mu\text{ghr/ml}$)
1	11	170	10	-24	4.80
2	12	210	10	-11	5.89
3	9	520	10	-35	7.73
Ref. Cpd.	5	500	10	-35	3.34

- 18 -

We claim:

1. A compound having the structural formula



- 5 or a pharmaceutically acceptable salt thereof, wherein X is chloro, fluoro or bromo.
2. A compound of claim 1 selected from:
 2'-[[4'-fluoro-[1,1'-biphenyl]-4-yl]methyl]-5'-methyl-spiro[cyclo-
 10 pentane-1,7'(8'H)-[3H]imidazo-[2,1-b]purin]-4'(5'H)-one;
 2'-[[4'-chloro-[1,1'-biphenyl]-4-yl]methyl]-5'-methyl-spiro[cyclo-
 pentane-1,7'(8'H)-[3H]imidazo-[2,1-b]purin]-4'(5'H)-one; and
 2'-[[4'-bromo-[1,1'-biphenyl]-4-yl]methyl]-5'-methyl-spiro[cyclo-
 15 pentane-1,7'(8'H)-[3H]imidazo-[2,1-b]purin]-4'(5'H)-one.
3. A pharmaceutical composition comprising an effective amount of a compound of claim 1 or 2 in a pharmaceutically acceptable carrier.
4. A process for the preparation of a pharmaceutical composition as
 20 defined in claim 3 comprising admixing a compound as claimed in claims 1 or 2 with a pharmaceutically acceptable carrier.
5. The use of a compound of claim 1 or 2 for the preparation of a
 25 medicament for treating hypertension, angina, bronchoconstriction, restenosis post angioplasty, atherosclerosis, ischemia, peripheral vascular diseases, or diseases benefitting from platelet inhibition, or for maintaining guanosine 3',5'-cyclic monophosphate (cGMP) levels.
6. A method of treating hypertension, angina, bronchoconstriction,
 30 restenosis post angioplasty, atherosclerosis, ischemia, peripheral vascular diseases, or diseases benefitting from platelet inhibition, or for maintaining guanosine 3',5'-cyclic monophosphate (cGMP) levels, comprising administering an effective amount of a compound of claim 1 or 2 to a mammal in need of such treatment.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C07D487/20 A61K31/505 A61K31/52		Int. l. Application No PCT/US 96/18550
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 19717 A (SCHERING CORPORATION) 26 December 1991 cited in the application see the whole document ---	1-6
A	WO 94 19351 A (SCHERING CORPORATION) 1 September 1994 cited in the application see the whole document -----	1-6
<div style="display: flex; justify-content: space-between;"> <input type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div>		
<div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents :</p> <p>* "A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>* "E" earlier document but published on or after the international filing date</p> <p>* "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>* "O" document referring to an oral disclosure, use, exhibition or other means</p> <p>* "P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>* "&" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search <div style="text-align: center; font-weight: bold;">4 March 1997</div>		Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">1 2. 03. 97</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016		Authorized officer <div style="text-align: center; font-weight: bold;">Luyten, H</div>

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/18550

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 6 is directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/18550

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9119717 A	26-12-91	AU 651607 B	28-07-94
		AU 8199291 A	07-01-92
		CA 2085733 A	22-12-91
		EP 0538332 A	28-04-93
		HU 65623 A	28-07-94
		JP 7047589 B	24-05-95
		KR 9604532 B	06-04-96
		NZ 238609 A	23-12-93
		OA 9723 A	30-08-93
		PL 169481 B	31-07-96
		US 5393755 A	28-02-95
WO 9419351 A	01-09-94	AU 6268794 A	14-09-94
		CA 2156918 A	01-09-94
		CN 1118600 A	13-03-96
		EP 0686157 A	13-12-95
		FI 953981 A	24-08-95
		HU 72640 A	28-05-96
		JP 8507068 T	30-07-96
		ZA 9401280 A	18-10-94

